

A novel histone deacetylase inhibitor prevents IL-1 β induced metabolic dysfunction in pancreatic β -cells

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Abstract

The histone deacetylase (HDAC) inhibitor trichostatin A (TSA) has recently been shown to inhibit deleterious effects of cytokines on β -cells, but it is unable to protect β -cells from death due to its own cytotoxicity. Herein, we investigated novel HDAC inhibitors for their cytoprotective effects against IL-1 β -induced damage to isolated β -cells. We report that three novel compounds (THS-73-44, THS-72-5 and THS-78-5) significantly inhibited HDAC activity and increased the acetylation of histone H4 in isolated β -cells. Further, these compounds exerted no toxic effects on metabolic cell viability in these cells. However, among the three compounds tested, only THS-78-5 protected against IL-1 β -mediated loss in β -cell viability. THS-78-5 was also able to attenuate IL-1 β -induced inducible nitric oxide synthase expression and subsequent NO release. Our data also indicate that the cytoprotective properties of THS-78-5 against IL-1 β -mediated effects may, in part, be due to inhibition of IL-1 β -induced transactivation of nuclear factor κ B (NF- κ B) in these cells. Together, we provide evidence for a novel HDAC inhibitor with a significant potential to prevent IL-1 β -mediated effects on isolated β -cells. Potential implications of these findings in the development of novel therapeutics to prevent deleterious effects of cytokines and the onset of autoimmune diabetes are discussed.

Keywords: HDAC inhibitors • pancreatic β -cell • inducible nitric oxide synthase • NO release • NF- κ B

Introduction

The acetylation status of core histones can greatly influence the rate of transcription of ~2% of genes. This cycle is controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Generally, acetylated histones allow a more open conformation and therefore increase the transcription of the DNA. The HATs acetylate ϵ -lysine residues on the tails of the core histones, whereas the HDACs facilitate the removal of those acetyl groups. At least three classes of HDACs consisting of 18 known isoforms have been identified thus far [1]. The first two classes consist of zinc-dependent HDACs 1–11, which are inhibited by known HDAC

inhibitors such as TSA, MS-275, as well as polyaminohydroxamic acids and polyaminobenzamides [2]. The third family of HDACs is the sirtuins, which are NAD⁺-dependent and resistant to zinc-binding HDACs. TSA, MS-275 and other zinc co-ordinating HDACs have many important functions, including the regulation of cell proliferation and inflammation [3–6]. Inhibitors of the class I and II HDACs are currently under investigation as therapeutic agents for many diseases, including cancer and rheumatoid arthritis [7–9]. As anti-cancer agents, HDAC inhibitors can induce growth arrest, differentiation and programmed cell death [3,5,6]. Also, HDAC inhibitors have been shown to exhibit anti-inflammatory effects in rheumatoid arthritis by inhibiting key transcription factors including nuclear factor κ B (NF- κ B) and signal transducer and activator of transcription (STAT; [10]).

Histone acetylation has also been shown to be important for islet β -cell function; specifically the acetylation of histone H4 has been shown to be critical for insulin gene transcription [11–13]. Emerging evidence appears to indicate that the acetylation status

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of specific histones plays a regulatory role in the induction of apoptosis in pancreatic β -cells. For example, using INS-1 cells and normal rat islets, Larsen *et al.* [14] have recently reported significant inhibition of cytokine-induced metabolic dysfunction by HDAC inhibitors, such as suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA). These investigators demonstrated that inhibition of HDACs markedly reduced cytokine-mediated inducible nitric oxide synthase (iNOS) expression, nitric oxide (NO) release and apoptosis in these cells [14]. They also provided further evidence to suggest that cytoprotective effects of HDAC inhibitors against cytokine-mediated effects may, in part, be due to down-regulation of NF- κ B activity. Along these lines, recent observations from our laboratory [15] have provided immunological evidence to suggest that Class 1 and 2 HDACs are localized in pancreatic islets and isolated β -cells. We also demonstrated a significant inhibition by TSA of IL-1 β -mediated iNOS expression and NO release in INS 832/13 cells; such effects of TSA appear to be mediated *via* increasing the acetylation status of histone H4. Together, data from the above studies support the hypothesis that hypoacetylation of specific histones (*e.g.* histone H4) favour the induction of iNOS under the duress of cytokines in isolated β -cells. The above-mentioned studies with TSA provided useful mechanistic details with respect to the prevention of cytokine-mediated effects on β -cells; however, they could pose significant problems for future studies since these inhibitors (*e.g.* TSA) are by themselves cytotoxic to the β -cell. Therefore, there is an immediate need for the development of specific HDAC inhibitors with limited cytotoxicity for potential therapeutic use in prevention of cytokine-induced damage to the β -cell.

In this context, Woster and colleagues have recently reported [2] synthesis of a series of polyaminobenzamides, which are isoform-selective HDAC inhibitors. In the current study, we report the ability of this class of HDAC inhibitors to attenuate HDAC activity and increase the acetylation status of specific histones in INS 832/13 cells (herein referred to as INS cells). We also report the efficacy of these inhibitors to attenuate IL-1 β -mediated iNOS expression, NO release and loss in metabolic cell viability. Potential implications of these findings in the development of novel therapeutics to prevent the deleterious effects of cytokines and the onset of autoimmune diabetes are discussed.

Materials and methods

Materials

IL-1 β was purchased from R&D Systems (Minneapolis, MN, USA), and Griess reagent, TSA and anti-actin serum were purchased from Sigma (St Louis, MO, USA). Cell viability assay (MTT) kit was purchased from Roche (Indianapolis, IN, USA). Affinity purified, monoclonal antibody directed against iNOS was purchased from Transduction Laboratories (Lexington, KY, USA). Antibodies directed against I κ B α and its phosphorylated form (Ser32/36) and total and acetylated H4 (acetyl-peptide corresponding to

residues surrounding Lys8 of histone H4) were purchased from Cell Signaling Technology, Inc (Danvers, MA, USA).

Synthesis of inhibitors

Novel polyaminobenzamide inhibitors were synthesized using previously described synthetic routes [2,16].

Cell culture

INS 832/13 (INS) cells were kindly provided by Dr. Chris Newgard (Duke University Medical Center, Durham, NC, USA) and were cultured in RPMI 1640 medium supplemented with 1 mM sodium pyruvate, 50 μ M β -mercaptoethanol and 10 mM HEPES at pH 7.4. The medium was changed twice weekly, and cells were trypsinized and subcloned weekly [17,18].

Quantitation of IL-1 β -induced NO release

INS cells were cultured in 24-well plates (3×10^5 cells/well) for 2 days prior to various experimental manipulations (see text). β -cells were then cultured in 500 μ L of medium/well containing either diluent alone or THS-78-5 (0–30 μ M) for 24 hrs. The culture medium was then replaced with 500 μ L of fresh medium containing THS-78-5 alone or in combination with IL-1 β (600 pM) for 24 hrs. The medium was collected after the incubation period and centrifuged at 100g for 5 min. Equal volumes (100 μ L) of medium and Griess reagent were mixed, and the absorbance was measured at 540 nm, as we previously described [19–21].

Quantitation of IL-1 β -induced iNOS expression

INS cells grown in 24-well plates were treated with THS-78-5 (0–30 μ M) for 24 hrs, prior to challenge with IL-1 β (600 pM) for 24 hrs as described above. Extracted proteins from different conditions were separated by SDS-PAGE, and then, resolved proteins were transferred to a nitrocellulose membrane by wet transfer as we described in References [19–21]. Blots were then probed with either antibody raised against iNOS or actin and further incubated with secondary antibody conjugated to horseradish peroxidase (HRP). Immune complexes were detected using an enhanced chemiluminescence (ECL) kit.

Quantitation of IL-1 β -induced phosphorylation of I κ B α

INS cells grown in 24-well plates were treated with diluent or THS-78-5 (0–30 μ M) for 24 hrs prior to challenge with IL-1 β (600 pM) for 0–120 min. Proteins were extracted in a buffer consisting of 20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β -glycerolphosphate, 1 mM Na₃VO₄, 1 μ g/ml leupeptin and 1 mM phenylmethylsulfonyl fluoride (PMSF). Extracted proteins from different conditions were separated by SDS-PAGE, and then, resolved proteins were transferred to a nitrocellulose membrane

by wet transfer as we described in References [19–21]. Membranes were then probed with an antibody raised against phosphorylated I κ B α and further incubated with secondary antibody conjugated to HRP. Immune complexes were detected using an ECL kit. The same membranes were further stripped and reprobed using antibodies raised against total I κ B α or actin.

Identification of acetylated histones

INS cells grown in 24-well plates were treated with THS-73–44 (10 μ M), THS-72–5 (10 μ M) or THS-78–5 (10 μ M) for 24 hrs as described above. Extracted proteins from different conditions were separated by SDS-PAGE, and then, resolved proteins were transferred to a nitrocellulose membrane by wet transfer, as we described in References [19–21]. Blots were then probed with an antibody raised against acetylated histone H4. Immune-complexes were detected using an ECL kit. The same membranes were further stripped and reprobed using rabbit polyclonal antibody raised against total histone H4 or actin.

Histone deacetylase activity assay

Nuclear extracts from INS cells were prepared using the NE-PER nuclear and cytoplasmic extraction kit from Pierce Biotechnology (Rockford, IL, USA). HDAC activity was measured using the HDAC assay kit from Upstate (Lake Placid, NY, USA). In brief, 20 μ g of nuclear extracts from INS cells or HeLa cells (positive control), as the source of HDAC activity, were incubated in the presence or absence of TSA (200 nM) or THS-78–5 (30 μ M) and acetylated substrate at 37°C for 60 min., allowing deacetylation of the colorimetric substrate. Subsequent addition of the activator solution after the incubation period selectively releases the colorimetric molecule from the deacetylated substrate, and the color developed was read at 405 nm. The HDAC activity was calculated as percentage of control in the absence of TSA or THS-78–5 (*i.e.* μ M of deacetylated substrate formed/min).

Metabolic cell viability determinations

INS cells were seeded at a density of 1×10^6 cells/ml in 96-well plates and then treated with diluent or THS-73–44 (0–10 μ M), THS-72–5 (0–10 μ M) or THS-78–5 (0–30 μ M) for 24 hrs, prior to the addition of IL-1 β (600 pM; 24 hrs). Cell viability was determined by a colorimetric assay (at 550–690 nm), performed with 3-[4,5-dimethylthiazolyl-2] 2,5-diphenyltetrazolium bromide (MTT), which measures the reduction of MTT into the blue formazan product by metabolically active cells.

Protein assay

Protein concentrations were determined by dye-binding method of Bradford, performed with bovine serum albumin as the standard [22].

Statistical analyses of data

Statistical significance of differences between the control and experimental groups was determined by ANOVA and a *P* value less than 0.05 was considered significant.

Results

Novel HDAC inhibitors increase the acetylation status of histone H4 in INS cells

It is well established that chronic exposure of pancreatic β -cells to cytokines (*e.g.* IL-1 β) culminates in β -cell death by either necrosis or apoptosis [23–25]. As stated in the Introduction, recent studies have clearly demonstrated that hypoacetylation of specific histones (*e.g.* histone H4) favours the induction of iNOS and subsequent NO release in isolated β -cells in the presence of cytokines (see introduction). In the present study, we investigated the potential beneficial effects of three novel HDAC inhibitors (Fig. 1) against IL-1 β -mediated effects in pancreatic β -cells. At the outset, we investigated regulatory effects of these three inhibitors (Fig. 1) on histone H4 acetylation in INS cells. Data in Figure 2A and B indicate that of the three compounds tested, THS-78–5 exerted maximal effects on histone H4 acetylation (610% of control). THS-73–44 and THS-72–5 demonstrated increases in acetylation of histone H4 of 160% and 440% of control, respectively.

Novel HDAC inhibitors markedly reduce HDAC activity in nuclear extracts-derived from INS cells or HeLa cells

We next quantitated the HDAC inhibitory property of THS-78–5 in INS nuclear extracts using a commercially available kit (see Methods for additional details). As a positive control, TSA was also included in these assays. Furthermore, the HDAC inhibitory property of this compound was also determined in nuclear extracts from HeLa cells. Data described in Figure 3 demonstrate a significant inhibition of HDAC activity by TSA in nuclear extracts from both HeLa (~83%) and INS cells (~77%). THS-78–5 also inhibited total HDAC activity, albeit to a lesser extent (~47%) in both INS and HeLa cell nuclear extracts. Not shown here are data to indicate significant inhibition of HDAC activity by THS-73–44 (~42%) and THS-72–5 (~67%) in HeLa cell nuclear extracts. Together, data in Figures 2 and 3 demonstrate that all three HDAC inhibitors significantly inhibit HDAC activity and promote the acetylation of histone H4 in pancreatic β -cells.

Of the three novel HDAC inhibitors tested, only THS-78–5 protects β -cells against IL-1 β -induced loss in metabolic cell viability

To address this, we first determined the cytotoxic effects, if any, of the three novel HDAC inhibitors (0–10 μ M) by assessing their effects on metabolic cell viability in INS cells (see Methods for additional experimental details). A modest, but significant increase (~10% and 14%, respectively) in the viability was noted in cells

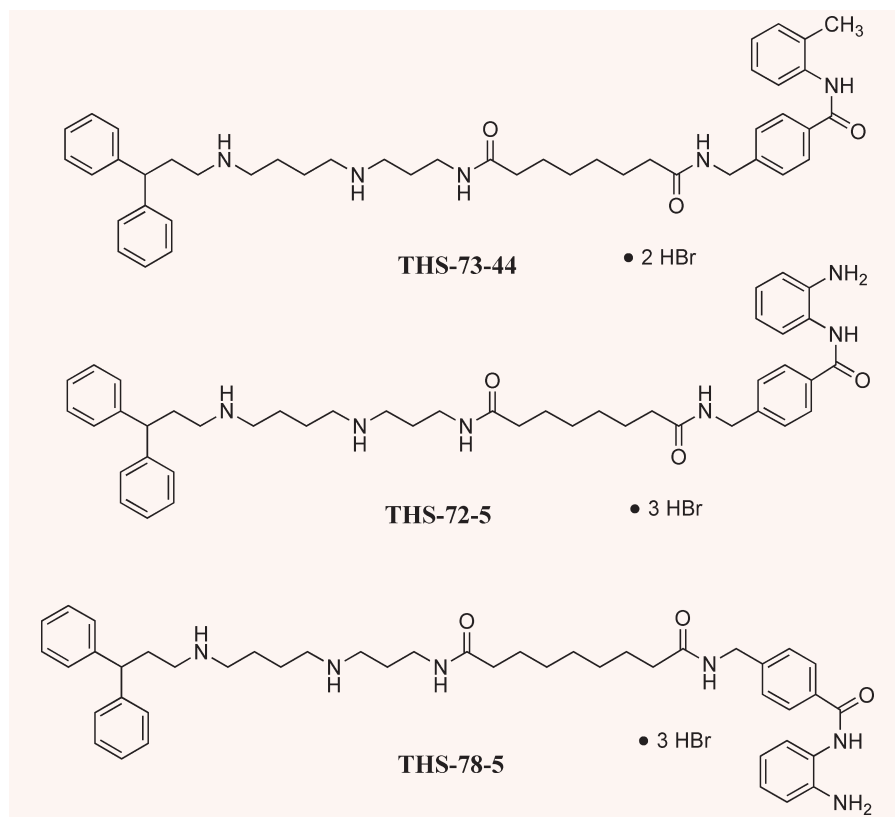


Fig. 1 Structures of polyaminobenza-mide histone deacetylase inhibitors THS-73-44, THS-72-5 and THS-78-5.

incubated in the presence of THS-73-44 (at both 5 and 10 μ M; Fig. 4). In contrast, a modest but significant reduction (~10%) in cell viability was noted in cells incubated with 10 μ M THS-72-5 (Fig. 4). No significant effects of THS-78-5 on metabolic cell viability were noted in cells treated with diluent alone (Fig. 4). As expected, treatment with IL-1 β resulted in a ~37% decrease in metabolic cell viability compared with cells treated with diluent alone (Fig. 4). Interestingly, however, only THS-78-5 (10 μ M) elicited significant protective effects against IL-1 β -induced loss in metabolic cell viability (bar 2 *versus* bar 12; Fig. 4). Data described in Figure 5 indicate that THS-78-5 alone had no cytotoxic effect in INS cells up to 30 μ M. As in Figure 4, cytoprotective effects of this compound against IL-1 β -induced loss in viability were demonstrable with concentrations as low as 10 μ M (Fig. 5).

THS-78-5 attenuates IL-1 β -induced iNOS expression and NO release in pancreatic β -cells

Since the above studies (Figs 1–5) clearly demonstrated a significant increase in acetylation of histone H4 and also a significant protection of cell viability by THS-78-5, we next investigated the regulatory (and potentially inhibitory) effects of THS-78-5 on IL-1 β -mediated increase in iNOS expression (Fig. 6A and B) and subsequent NO release (Fig. 6C). Data from these studies indicated

that maximal inhibition of IL-1 β -induced iNOS expression was demonstrable by THS-78-5 at 30 μ M (~67% inhibition). Likewise, maximal inhibition of NO release was seen at 30 μ M THS-78-5 (Fig. 6C). Taken together, these data indicate that THS-78-5 prevents IL-1 β -mediated effects on iNOS expression, NO release and metabolic cell viability in INS cells in a manner akin to TSA. More importantly, however, this compound, unlike TSA, elicits no cytotoxic effects as demonstrated by no significant effects on the metabolic cell viability of the cells.

THS-78-5 attenuates IL-1 β -mediated toxic effects on isolated β -cells by inhibiting transactivation of NF- κ B

Since recent studies by Larsen *et al.* [14] have identified NF- κ B as one of the possible loci for the action of HDAC inhibitors (*e.g.* TSA) in isolated β -cells, we investigated, herein, potential regulatory effects of THS-78-5 on I κ B α phosphorylation and degradation in INS cells following short-term incubation with IL-1 β . As shown in Figure 7, THS-78-5 significantly decreased IL-1 β -induced phosphorylation of I κ B α , following 15 and 30 min. of challenge with IL-1 β . Further, compatible with earlier observations performed with TSA [14], it also reduced the resynthesis of I κ B α at all time-points studied (Fig. 7). In the absence of IL-1 β ,

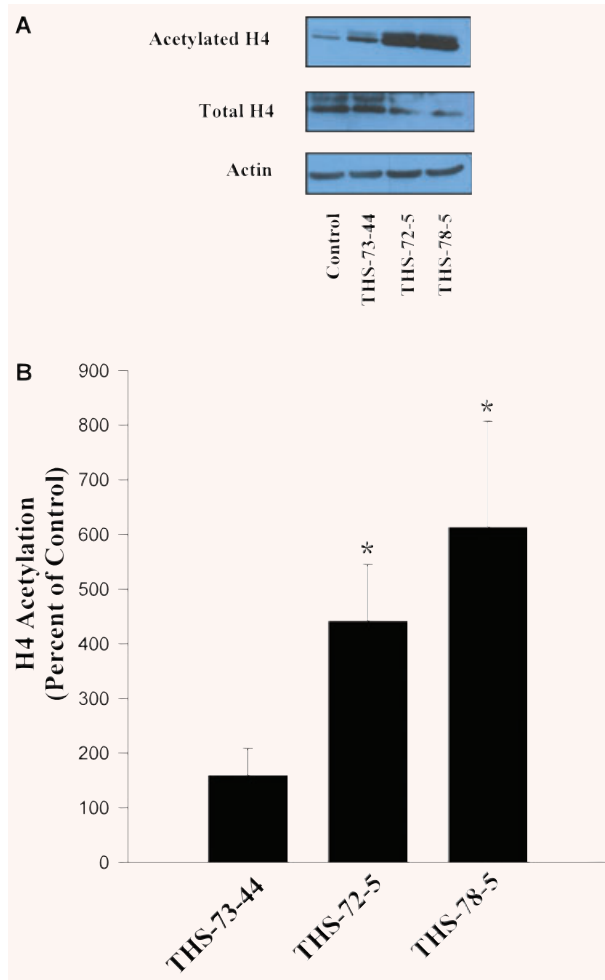


Fig. 2 Novel HDAC inhibitors increase the acetylation of histone H4 in INS cells: **(A)** INS cells were incubated in the presence or absence of THS-73-44 (10 μ M), THS-72-5 (10 μ M) or THS-78-5 (10 μ M) for 24 hrs. Lysates were separated by SDS-PAGE, transferred onto nitrocellulose membranes and subjected to immunoblot analysis with an antibody directed against acetylated H4, total H4 or actin, followed by incubation with an HRP conjugated secondary antibody for 1 hr. Immune complexes were detected using an ECL kit. A representative blot from three experiments yielding similar results is shown. **(B)** Relative intensities of the acetylated H4 bands were quantified by densitometry and plotted as percent of acetylated H4 in the presence of diluent alone. Data are mean \pm SEM from three independent experiments. * P < 0.05 versus control cells.

THS-78-5 had no effect on I κ B α phosphorylation or degradation (additional data not shown). Together, these findings support the overall hypothesis that HDAC inhibitors like TSA [14] and THS-78-5 inhibit IL-1 β -mediated effects on isolated β -cells, possibly by interfering with the transactivation of NF- κ B and subsequent down-stream signaling events (see Discussion).

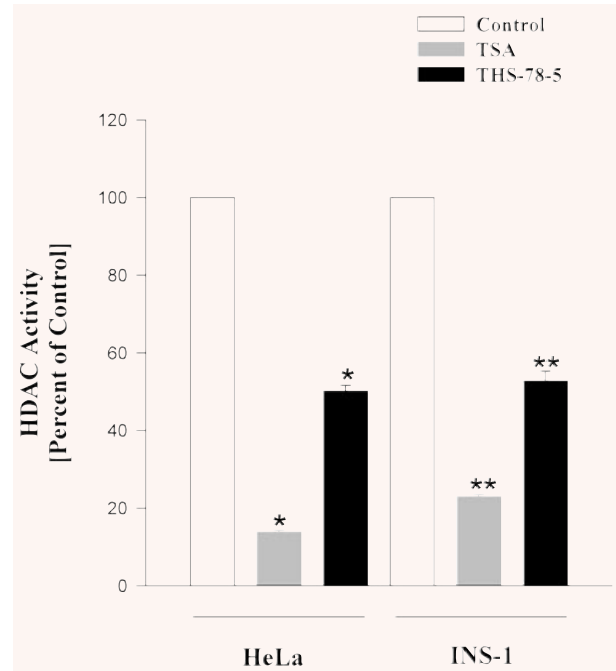


Fig. 3 THS-78-5 significantly inhibits HDAC activity in INS nuclear extracts: nuclear extracts from INS cells were prepared using the NE-PER nuclear and cytoplasmic extraction kit (see Methods for additional details). In brief, 20 μ g of nuclear extracts from INS cells or HeLa cells (used as a positive control) were incubated in the presence or absence of TSA (200 nM) or THS-78-5 (30 μ M) and the degree of deacetylation of the substrate was measured colorimetrically at 405 nm using a commercially available kit (see Methods for additional details). The data, which are expressed as percentage of HDAC activity in untreated controls, are mean \pm SEM from three independent experiments. * P < 0.05 versus control, untreated nuclear extracts purified from HeLa cells. ** P < 0.05 versus control, untreated nuclear extracts purified from INS cells.

Discussion

One of the major aims of the current investigation was to increase our current understanding of epigenetic control mechanisms (*e.g.* histone [de]acetylation) underlying cytokine-mediated metabolic dysfunction of the pancreatic β -cell. Salient features of our study are: (i) three novel compounds (THS-73-44, THS-72-5 and THS-78-5; synthesized in our laboratories) inhibited HDAC activity and increased the acetylation of histone H4 in isolated β -cells; (ii) they further exerted no toxic effects on metabolic cell viability in these cells; (iii) however, of the compounds tested, only THS-78-5 protected against IL-1 β -mediated loss in cell viability; (iv) THS-78-5 was also able to attenuate IL-1 β -induced iNOS expression and subsequent NO release; and (v) cytoprotective properties of THS-78-5 against IL-1 β -mediated effects may, in part, be due to inhibition of IL-1 β -induced transactivation of NF- κ B in these cells. Together, we

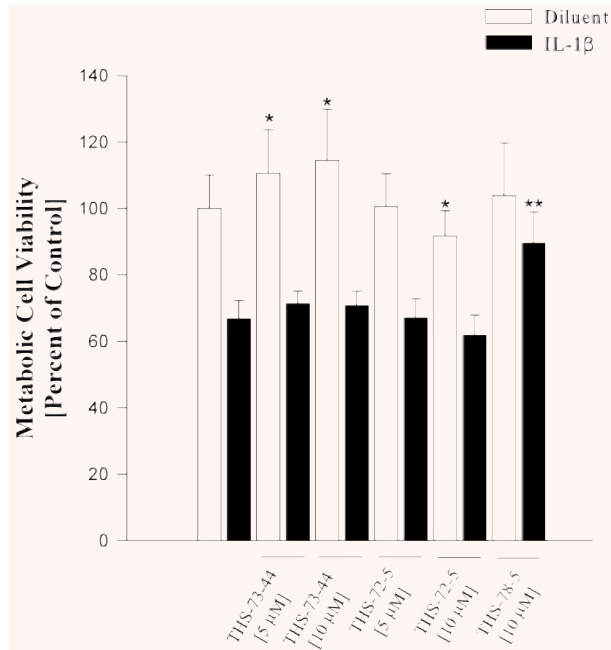


Fig. 4. Novel HDAC inhibitors attenuate the IL-1 β -induced decrease in metabolic viability: INS cells were incubated in the absence or presence of IL-1 β (600 pM; 24 hrs). THS-73-44 (0–10 μ M), THS-72-5 (0–10 μ M) or THS-78-5 (10 μ M) was also present in the incubation medium. Metabolic cell viability was determined using the MTT assay as described in Methods. Data are expressed as percentage of control (mean \pm SEM) from three independent studies. * P < 0.05 compared with control, untreated cells. ** P < 0.05 compared with cells treated with IL-1 β alone.

provide evidence for a novel HDAC inhibitor with a significant potential to prevent cytokine-mediated effects on isolated β -cells.

Emerging evidence from multiple laboratories appears to implicate chromatin structure remodelling in gene transcription [26,27]. One such structural modification of chromatin is through the acetylation of histones, the major protein components of chromatin [28]. This type of modification facilitates the transcription of otherwise restricted genes. Several lines of recent evidence also suggest that a number of transcriptional factors are tightly regulated by histone modification [29–31], including the transactivation of NF- κ B [32–34], which has been shown to promote the induction of iNOS in multiple cells lines, including pancreatic β -cells under the duress of various inflammatory stimuli including cytokines [14,35–39]. However, experimental evidence on the precise roles of the acetylation of histones and/or the regulation of acetylated histones in pancreatic β -cells in the context of IL-1 β -induced iNOS expression and subsequent NO release has emerged only recently [14,15].

In a study published in 2007, Larsen *et al.* [14] presented the first evidence to indicate that inhibition of HDACs prevents cytokine-induced toxicity in pancreatic β -cells. Using two structurally distinct HDAC inhibitors (*e.g.* TSA and SAHA), they reported that HDAC inhibition leads to inhibition of cytokine-

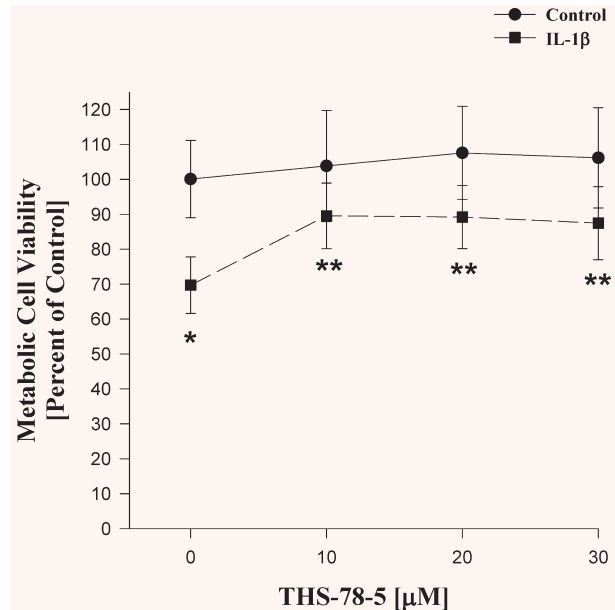


Fig. 5 THS-78-5 attenuates the IL-1 β -induced decrease in metabolic cell viability: INS cells were incubated in the absence or presence of IL-1 β (600 pM; 24 hrs) as indicated in the figure. THS-78-5 (0–30 μ M) was also present in the incubation medium. Metabolic cell viability was determined using MTT assay as described in Methods. Data are expressed as percentage of control (mean \pm SEM) from three independent studies. * P < 0.05 compared with control, untreated cells. ** P < 0.05 compared with cells treated with IL-1 β alone.

induced iNOS expression and NO release in INS-1 cells and normal rat islets. They also demonstrated protection of cytokine-induced inhibition of insulin secretion by HDAC inhibitors. Further, they were able to show that HDAC inhibitors markedly down-regulate the transactivation of NF- κ B induced by cytokines. In a more recent study from our laboratory, we have provided further support to Larsen *et al.*'s observations [15]. First, we provided immunological evidence for the localization of Class 1 and 2 HDACs in isolated β -cells. Second, using INS cells, we were able to demonstrate a marked increase in the acetylation of histone H4 and reduction in IL-1 β -induced iNOS expression and NO release by TSA in these cells. Together, these data suggest that hyperacetylation of histone H4 leads to inhibition of cytokine-induced iNOS expression and NO release.

Yu and coworkers provided evidence to suggest that HDACs augment cytokine-mediated induction of iNOS gene expression and subsequent NO release in cultured murine mesangial cells and RAW 264.7 cells [38]. Using TSA, they observed a marked reduction in IL-1 β - or lipopolysaccharide plus interferon γ -induced iNOS expression, NO release and NF- κ B promoter activity. They were able to further demonstrate potentiation of IL-1 β -mediated effects in cells overexpressing specific HDACs. Together, these findings provided conclusive evidence to implicate histone

acetylation–deacetylation in cytokine-induced effects in renal cells. More recent studies by these investigators [40] have reported that TSA-mediated reduction in IL-1 β -induced effects involves additional signaling mechanisms, such as phosphoinositide 3-kinase and p70s6 kinase, which increase site-specific histone H4 acetylation at the –978 to –710 region of the iNOS promoter.

The data presented in this manuscript clearly demonstrates that of the three analogues evaluated, the polyaminobenzamide THS-78-5 produces the greatest increase in histone H4 acetylation and most effectively attenuates cytokine-mediated cytotoxic effects. *In vitro* HDAC inhibition studies reveal that THS-72-5 is the most potent of the three with respect to global HDAC inhibition. However, in studies involving the pure HDAC isoforms 1, 3, 6 and 8, THS-78-5 appears to be most selective for HDAC 1 (data not shown). The significance of this observation in the present study is as yet unknown. It is not possible to make meaningful structure/function correlations from a library of only three compounds, but it is obvious that small changes in structure have a great effect on the ability of polyaminobenzamides to protect β -cells from IL-1 β -mediated damage. Compound THS-73-44 is identical to THS-72-5, except for an amino group to methyl group structural change. The diminished effects of THS-73-44 could be attributed to a reduced ability of the benzamide moiety to co-ordinate with the zinc ion in the HDAC active site. The difference in activity between THS-72-5 and THS-78-5 is more difficult to explain since these compounds differ by a single methylene group in the linker chain portion of the molecule. Since the length of the linker chain has a direct bearing on the binding of polyaminobenzamides to the HDAC active site [2,16], it is likely that the seven carbon linker chain fits the active site channel more effectively than the six carbon linker, thus producing greater inhibition. Additional analogues will need to be evaluated in the system described in this manuscript before any meaningful structure/activity relationships for the observed effects can be inferred. It should also be noted that the concentration of polyaminobenzamides are much higher than that of TSA to inhibit the IL-1 β -induced effects, this is also true of other benzamide containing HDAC inhibitors (*i.e.* MS-275). This difference in the potencies between polyaminobenzamides and TSA may be due to difference in the ability to inhibit different subsets of HDACs.

In summary, based on findings in multiple cell types, including the islet β -cell [14,15,38,40], we propose that hyperacetylation of histone H4 is necessary for the attenuation of cytokine-mediated cytotoxic effects including iNOS expression, NO release and eventual loss in cell viability. It also appears that HDAC inhibitors

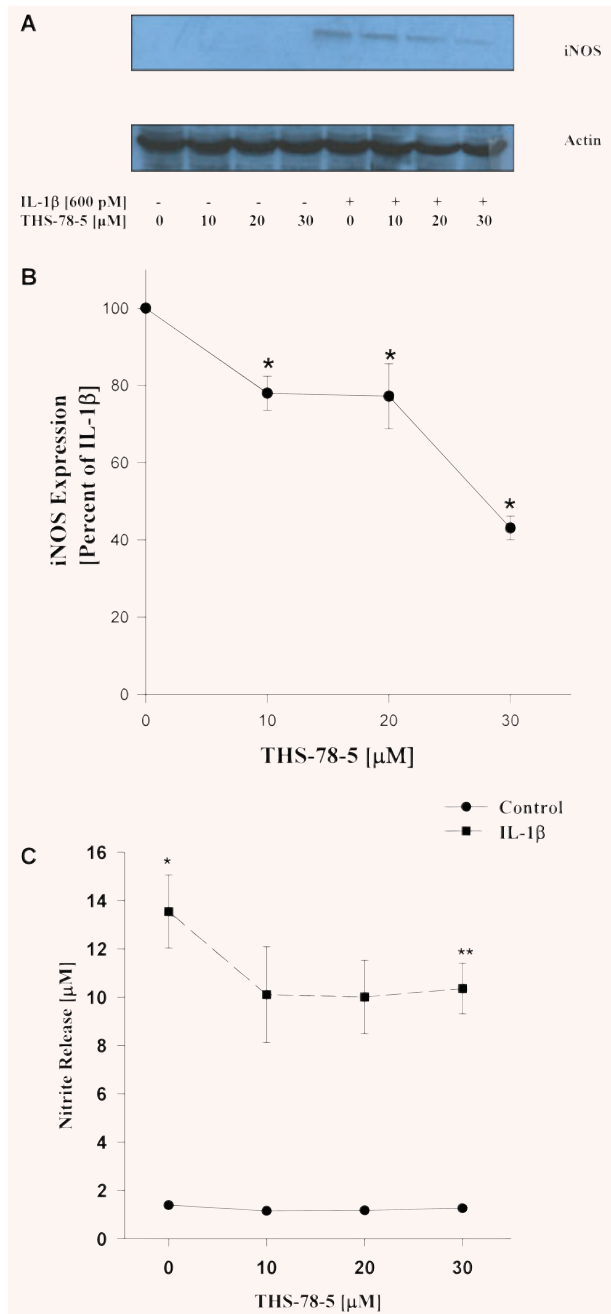


Fig. 6 THS-78-5 attenuates IL-1 β -induced NO release and iNOS expression in INS cells. **(A)** INS cell lysates incubated in the absence or presence of IL-1 β (600 pM; 24 hrs) and THS-78-5 (0–30 μ M) were separated by SDS-PAGE, transferred onto nitrocellulose membranes and subjected to immunoblot analysis with an antibody directed against iNOS or actin. A representative blot from three experiments yielding similar results is shown. **(B)** Relative intensities of the iNOS bands were quantified by densitometry and plotted as percentage inhibition of iNOS expression compared with IL-1 β treated cells. Data are mean \pm SEM from three independent experiments. * P < 0.05 compared with cells treated with IL-1 β alone. **(C)** INS cells were incubated in the absence or presence of IL-1 β (600 pM; 24 hrs). THS-78-5 (0–30 μ M) was also present in the incubation medium (24 hrs prior to the addition of IL-1 β). NO released into the medium was quantitated using Griess reagent. Data, which are expressed as NO released into the medium, are mean \pm SEM from three independent experiments carried out in triplicate. * P < 0.05 compared with basal NO release in the absence of IL-1 β . ** P < 0.05 compared with NO release in the presence of IL-1 β alone.

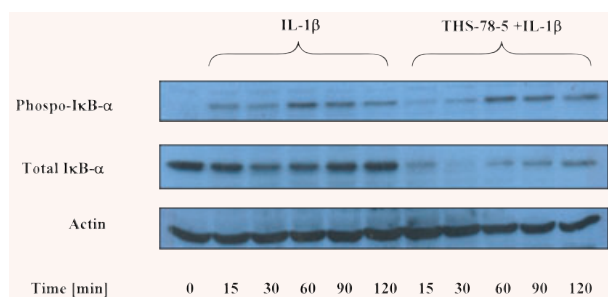


Fig. 7 Effects of IL-1 β and THS-78-5 on I κ B α phosphorylation and degradation in INS cells: INS cells were incubated in the presence or absence of THS-78-5 (30 μ M), for 24 hrs prior to challenge with IL-1 β (600 pM) for 0–120 min. Lysates were separated by SDS-PAGE, transferred onto nitrocellulose membranes and subjected to immunoblot analysis with an antibody directed against phosphorylated I κ B α , total I κ B α or actin, followed by incubation with an HRP conjugated secondary antibody for 1 hr. Immune complexes were detected using an ECL kit. A representative blot from three experiments yielding similar results is shown.

clearly act through inhibition of transactivation of NF- κ B. Our current findings of identification of THS-78-5 as one of the lead compounds to prevent cytokine-mediated metabolic dysfunction of the islet β -cell are of high translational value since this series of compounds, unlike other known HDAC inhibitors (*e.g.* TSA or SAHA), is by itself not cytotoxic. Studies for the development of additional structural analogues with much lower IC₅₀ values to inhibit islet β -cell specific HDACs are in progress in our laboratories.

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